

host cells include bacterial cells, such as *E. coli*, *B. thuringiensis*, *B. subtilis*, *B. megaterium*, or *Pseudomonas* spp. cells, with *B. thuringiensis* NRRL B-21590, NRRL B-21591, NRRL B-21592, NRRL B-21638, NRRL B-21639, NRRL B-21640, NRRL B-21609, and NRRL B-21610 cells being highly preferred. Another preferred host cell is an eukaryotic cell such as a fungal, animal, or plant cell, with plant cells such as grain, tree, vegetable, fruit, berry, nut, grass, cactus, succulent, and ornamental plant cells being highly preferred. Transgenic plant cells such as corn, rice, tobacco, potato, tomato, flax, canola, sunflower, cotton, wheat, oat, barley, and rye cells are particularly preferred.

Host cells which produce one or more of the polypeptide having insecticidal activity against Lepidopterans, host cells which are useful in preparation of recombinant toxin polypeptides, and host cells used in the preparation of a transgenic plant or in generation of pluripotent plant cells represent important aspects of the invention. Such host cells may find particular use in the preparation of an insecticidal polypeptide formulation, such as a polypeptide that comprises the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:59, or SEQ ID NO:61, and which is insecticidally active against Lepidopterans.

A polypeptide composition such as those described herein are particularly desirable for use in killing an insect cell, and in the preparation of an insecticidal formulation, such as a plant protective spray formulation. The polypeptide composition may be prepared by culturing a *B. thuringiensis* NRRL B-21590, NRRL B-21591, NRRL B-21592, NRRL B-21638, NRRL B-21639, NRRL B-21640, NRRL B-21609, or NRRL B-21610 cell under conditions effective to produce a *B. thuringiensis* crystal protein; and obtaining the *B. thuringiensis* crystal protein from the cell.

The polypeptide may be used in a method of killing an insect cell. This method generally involves providing to an insect cell an insecticidally-effective amount of the polypeptide composition. Typically, the insect cell is comprised within an insect, and the insect is killed by ingesting the composition directly, or alternatively by ingesting a plant coated with the composition, or ingesting a transgenic plant which expresses the polypeptide composition.

Another important embodiment of the invention is a purified antibody that specifically binds to a polypeptide having the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:59, or SEQ ID NO:61. Such antibody compositions may be operatively attached to a detectable label, or comprised within an immunodetection kit. Such antibodies find particular use in methods for detecting an insecticidal polypeptide in a biological sample. The method generally involves contacting a biological sample suspected of containing such a polypeptide with an antibody under conditions effective to allow the formation of immunocomplexes, and detecting the immunocomplexes so formed.

A transgenic plant having incorporated into its genome a transgene that encodes a polypeptide comprising the amino sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:59, or SEQ ID NO:61 also represents an important embodiment of the present invention. Such a transgenic plant preferably comprises the nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:58, or SEQ ID NO:60. Progeny and seed from such a plant and its progeny are also important aspects of the invention.

A method of selecting a Cry1 polypeptide having increased insecticidal activity against a Lepidopteran insect comprising mutagenizing a population of polynucleotides to prepare a population of polypeptides encoded by said polynucleotides and testing said population of polypeptides and identifying a polypeptide having one or more modified amino acids in a loop region of domain 1 or in a loop region between domain 1 and domain 2, wherein said polypeptide has increased insecticidal activity against said insects.

Another important embodiment of the invention is a method of generating a Cry1 polypeptide having increased insecticidal activity against a Lepidopteran insect. Such a method generally involves identifying in such a polypeptide a loop region between adjacent α -helices of domain 1 or between an α -helix of domain 1 and a β strand of domain 2, then mutagenizing the polypeptide in at least one or more amino acids of one or more of the identified loop regions; and, finally, testing the mutagenized polypeptide to identify a polypeptide having increased insecticidal activity against a Lepidopteran pest.

A method of mutagenizing a Cry1 polypeptide to increase the insecticidal activity of the polypeptide against a Lepidopteran insect is also provided by the invention. This method comprises predicting in such a polypeptide a contiguous amino acid sequence encoding a loop region between adjacent α -helices of domain 1 or between an α -helix of domain 1 and a β strand of domain 2; mutagenizing one or more of these amino acid residues to produce a population of polypeptides having one or more altered loop regions; testing the population of polypeptides for insecticidal activity against Lepidopterans; and identifying a polypeptide in the population which has increased insecticidal activity against a Lepidopteran insect.

In such methods, the modified amino acid sequence preferably comprises a loop region between α helices 1 and 2a, α helices 2b and 3, α helices 3 and 4, α helices 4 and 5, α helices 5 and 6, or α helices 6 and 7 of domain 1, or between α helix 7 of domain 1 and β strand 1 of domain 2. Preferably, the loop region between α helices 1 and 2a comprises an amino acid sequence of from about amino acid 41 to about amino acid 47 of a Cry1 protein. Likewise, the loop region between α helices 2b and 3 comprises an amino acid sequence of from about amino acid 83 to about amino acid 89 of a Cry1 protein, and the loop region between α helices 3 and 4 comprises an amino acid sequence of from about amino acid 118 to about amino acid 124 of a Cry1 protein. The loop region between α helices 4 and 5 preferably comprises an amino acid sequence of from about amino acid 148 to about amino acid 156 of a Cry1 protein, while the loop region between α helices 5 and 6 comprises an amino acid sequence of from about amino acid 176 to about amino acid 85 of a Cry1 protein. The loop loop region between α helices 6 and 7 preferably comprises an amino acid sequence of from about amino acid 217 to about amino acid 222 of a Cry1 protein, while the loop region between α helix 7 of domain 1 and β strand 1 of domain 2 preferably comprises an amino acid sequence of from about amino acid 249 to about amino acid 259 of a Cry1 protein.

Exemplary Cry1 proteins include Cry1A, Cry1B, Cry1C, Cry1D, Cry1E, Cry1F, Cry1G, Cry1H, Cry1I, Cry1J, and Cry1K crystal proteins, with Cry1Aa, Cry1Ab, Cry1Ac, Cry1Ad, Cry1Ae, Cry1Ba, Cry1Bb, Cry1Bc, Cry1Ca, Cry1Cb, Cry1Da, Cry1Db, Cry1Ea,